

FILE 'BIOSIS, MEDLINE, EMBASE, EMBAL, SCISEARCH, BIOTECHDS, CAPLUS'  
ENTERED AT 16:20:27 ON 05 AUG 2003

L1 89876 S (EBV OR (EPSTEIN (1W) BARR (1W) VIRUS?))

L2 3957 S L1 AND VECTOR?

L3 436 S L2 AND (PCR OR (AMPLIF?))

L4 75 S L3 AND (PRIMER?)

L5 56 DUP REM L4 (19 DUPLICATES REMOVED)

L6 5 S L5 AND PY<1993

L6 ANSWER 3 OF 5 BIOTECHDS COPYRIGHT 2003 THOMSON DERWENT/ISI  
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ACCESSION NUMBER: 1992-03039 BIOTECHDS

TITLE: A general and fast method to generate multiple site directed  
mutations;  
site-directed mutagenesis method using the polymerase  
chain reaction

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CODEN: NARHAD

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LANGUAGE: English

SO Nucleic Acids Res.; (1992) 20, 2, 376

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AB. . . method to generate multiple site-directed mutations (deletion,  
insertion or substitution) in a given DNA fragment. The method required  
3 universal primers for the vector, and only 1  
specific primer for each mutation. The method consisted of 2  
successive rounds of polymerase chain reaction (PCR). The 1st  
round consisted of 2 simultaneous PCRs, with primers (1 and 2)  
homologous to the vector, but with a mismatched 3'-end in  
primer 2. The 2nd PCR was done with primer 3  
and primer M (with a mutation). Amplified fragments  
were purified, mixed and subjected to another PCR round with  
external primers 1 and 3 (with no amplification of  
parental and hybrid B fragments). Only hybrid A was amplified,  
and since the 3'-end of primer 2 was not complementary to the  
DNA, only the mutated strand was amplified. The  
amplified fragment could then be cleaved and ligated into a  
vector. The method was used to generate 10 different deletion  
and substitution mutants in Epstein-Barr  
virus transcription factor EB1 or Z, with a routine efficiency of  
90%. (3 ref)

L Number	Hits	Search Text	DB	Time stamp
1	424	(EBV (epstein adj1 barr adj1 virus)) SAME (detection)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/05 17:02
2	217	((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/05 17:02
3	190	((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)) and (pcr or amplifi\$7)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/05 17:02
4	154	((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)) and (pcr or amplifi\$7)) and primer	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/05 17:03
5	12	((EBV (epstein adj1 barr adj1 virus)) SAME (detection)) and (vector)) and (pcr or amplifi\$7)) and primer) and (EBV (epstein adj1 barr adj1 virus)).ti.	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/05 17:03

L Number	Hits	Search Text	DB	Time stamp
1	12	(EBV (epstein adj1 barr adj1 virus)) and ((vca adj1 p18) (vca adj1 p40))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/04 15:42
2	10	((EBV (epstein adj1 barr adj1 virus)) and ((vca adj1 p18) (vca adj1 p40))) and (bfrf3 bdrf1)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/04 15:42
3	17	(EBV (epstein adj1 barr adj1 virus)) and (BFRF3 BdRF1)	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/04 15:42
4	7	((EBV (epstein adj1 barr adj1 virus)) and (BFRF3 BdRF1)) not (((EBV (epstein adj1 barr adj1 virus)) and ((vca adj1 p18) (vca adj1 p40))) and (bfrf3 bdrf1))	USPAT; US-PGPUB; EPO; JPO; DERWENT; IBM_TDB	2003/08/04 15:43